[20], later in life. Interestingly, the onset of senescence in giant dog breeds appeared to occur at a time at which these animals were still growing.

The paper by Kraus *et al.* [12] tells us why big dogs die young: a St Bernard ages more rapidly following the onset of senescence than a Pekinese does. We now need to focus in on potential mechanisms driving these differences. On a more cautionary note, dogs are rather peculiar given that they have been artificially selected for phenotypic diversity, in stark contrast to more routine model organisms primarily selected for similarity. However, there is no doubting that experimental approaches leading on from the work by Kraus *et al.* will complement those studying ageing using classical and non-classical model organisms in the laboratory, and under both semi-natural and natural conditions. We suggest that comparative approaches both across and within species are likely to give key insights into what mechanisms underlie the ageing process.

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Insect Biotechnology: Controllable Replacement of Disease Vectors

To fight human vector-borne diseases, first releases of sterile transgenic mosquitoes have been performed. Someday, disease-refractory mosquitoes will replace wild types to stop transmission. For such population replacements, gene drive mechanisms must be established that allow local confinement and reversibility.

Ernst A. Wimmer

The fight against Malaria and Dengue — the two major insect-borne diseases in tropical and subtropical areas — is threatened by the increasingly fast evolution of insecticide resistance in its insect vectors. Therefore, alternative control tools need to be included into pest management strategies [1]. Insect transgenesis promises to provide such novel tools through the establishment of conditional reproductive sterility or the refractoriness to disease transmission [2,3]. In the transmission of vector-borne diseases, the insect is only a nuisance, but does not actually cause the illness itself. This has led to the long-standing hope that wild mosquito populations could actually be replaced by biotechnologically engineered strains that would be refractory to the disease causing pathogens, such as protists or viruses [4,5], and therefore interrupt disease transmission [3]. As the simple replacement of a complete insect population by a desired strain is not feasible, strategies need to be developed that cause population replacement by changing the genetic make-up of the population through spreading the required refractoriness-causing transgenes. However, the transgenes providing refractoriness to human disease transmission are not likely to spread through the population by themselves. This requires effective gene drive mechanisms that allow for non-Mendelian inheritance and 'selfish genes' are thought to be some of the best vehicles for such a gene drive [6]. Conversely, there are concerns on how to contain such selfish genes and the accompanied

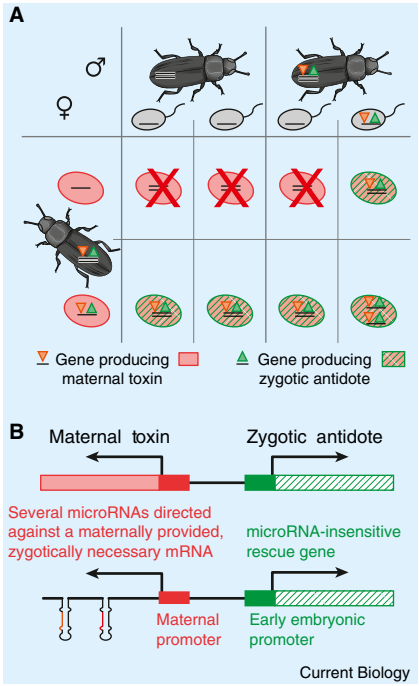

Figure 1. Maternal Effect Dominant Embryonic Arrest (MEDEA).

(A) MEDEA as originally described in *Tribolium castaneum* [17] providing a still hypothetical maternal lethal activity (maternal toxin) that kills all progeny not bearing the correct *Medea* allele and an embryonic rescue activity (zygotic antidote) protecting progeny that carry the *Medea* allele from the maternal lethal effect [18]. The death (red X) of the embryos not carrying the *Medea* allele causes a non-Mendelian. (B) Synthetic MEDEA composed of maternally expressed microRNAs against a maternally provided but embryonic necessary mRNA and a zygotic rescue gene, which is driven by an early embryonic promoter and insensitive to the microRNAs, thus providing the necessary gene function to restore the maternal-driven gene knock-out [19].

transgene spread, for instance by restricting them locally or by removing them once they are no longer needed due to resistance development [7]. In a recent study [8] published in *Current Biology*, the group of Bruce Hay has established a novel synthetic gene drive mechanism for local application and potential reversibility in *Drosophila melanogaster*.

The first biotechnologically engineered designer mosquitoes to fight the Dengue vector *Aedes aegypti* have already been released in small scale trials in the Grand Cayman Islands [9], Malaysia, and are currently released in Brazil [10]. The present control effort is based on a very successful genetic pest management strategy, termed 'sterile insect technique' (SIT). SIT has proven to prevent, control, suppress, or even eradicate invasive insect pest species — such as the Mediterranean Fruit Fly, the New World Screw Worm, or the Tsetse Fly — from islands, large agricultural production areas, or even complete continents [11]. SIT is essentially an insect birth control measure that involves mass production of the pest insects, their sterilization and sustained release of large quantities of sterilized insects over wide areas. When sterile insects mate with the native population, the unproductive matings shrink the population. Due to its species-specificity, SIT is considered an environmentally friendly pest management measure [12]. To use SIT for mosquito control, there are three features that insect transgenesis could improve on [13]: first, in mosquitoes only males can be released as even sterile females would blood-feed and could increase disease transmission rates. Transgenic female-specific lethality systems address this problem and could support efficient genetic sexing and subsequent male-only releases [14]. Second, conventional sterilization is carried out by irradiation that reduces at the same time the competitive fitness of released males. In this respect, transgenic systems that cause lethality after transmission to the progeny could provide reproductive sterility without fitness costs [15]. Third, transgenic marker systems will enable discrimination of released and naturally occurring insects, helping to monitor the efficacy of SIT applications, which is important in order to release the right amount of sterile insects for effective control while keeping costs down [13].

Reproductive sterility based on lethality systems serves as an intrinsic containment against vertical transmission of transgenes in biotechnologically engineered SIT and its application does, therefore, not present real concerns with respect to humans or the environment [13]. However, transgenic SIT is still at its very beginnings and its large scale use may be somewhat premature, especially as it might impinge on the future use of this technology due to possible development of resistance [16]. Despite the fact that SIT approaches have shown to be very successful for agricultural insect pests [11] and will probably be useful also to control human disease vectors, the insect in the transmission of vector-borne diseases is only annoying but not the cause of the illness itself. Therefore, replacement strategies have been considered that would not suppress or eradicate the insect vector but instead make it refractory to the transmission of the disease — causing pathogens [3]. To locally restrict the spread of or actually recall the refractoriness providing transgenes, the group of Bruce Hay has successfully tested a controllable synthetic gene drive mechanism based on the principle of underdominance using an artificial MEDEA (maternal effect dominant embryonic arrest) system in the insect model *Drosophila melanogaster* [8].

Maternal-effect selfish genes were first described in the red flour beetle *Tribolium castaneum* and called MEDEA [17] — a fitting acronym as in Greek mythology Medea killed her kids sired by the 'wrong' man, Jason, after he rejected her. *Tribolium Medea* elements are based on a still hypothetical maternal lethal activity that kills all progeny not bearing the correct *Medea* allele [18]. As this 'selfish' mechanism ensures that progeny of carrier mothers only survive when they inherit from either parent a copy of this genetic element, the inheritance is non-Mendelian (Figure 1A) and leads to a population spread of the genetic element. Based on this concept, a synthetic MEDEA system was designed in *Drosophila* [19] using maternally expressed microRNAs against a maternally provided mRNA that is necessary for embryo survival. The zygotic rescue activity in this synthetic system is due to a transgene from which the respective mRNA is transcribed and which is insensitive to the microRNAs (Figure 1B). This system drives population replacement by spreading itself in a non-Mendelian but uncontrollable manner [19]. Hay and colleagues [8] have further developed this synthetic system into double-MEDEA (Figure 2A) to get control over the transgene spread by under- or overdominance.

Underdominance (heterozygote inferiority) means that the fitness of both homozygous genotypes is higher than of the heterozygous genotype [20]. Over time, this leads to disruptive selection towards either of the two

divergent homozygous genotypes depending on the respective allele frequency. Underdominance systems are therefore preferential for local replacements with limited spread due to the high number of insects that would need to be released [6]. To generate underdominance, Hay and colleagues [8] use a two gene loci double-MEDEA system that is composed of two reciprocal maternal toxin–zygotic antidote transgene pairs that are placed at different positions in the genome (Figure 2A). When both transgene pairs are carried by the mother and thus both toxins are produced, both gene loci need to be inherited by the progeny for survival. Thus, all progeny of a wild type or a double homozygote cross but only about half of the progeny of a double heterozygote cross will survive, causing an underdominance effect (Figure 2C) that serves as an unstable bottleneck that will ultimately lead to divergent genotypes: either towards wild type or towards homozygous double transgenics depending on the initial number of wild-type or transgene carrying alleles of the gene loci, respectively [8]. Therefore, the underdominance effect can be used as a switch to either replace animals in a local population with transgene carriers (drive in, replacement) or to replace transgene carriers with wild-type animals (drive out, transgene recall, reversion) [8]. Transgenic underdominance systems are usually difficult to generate, because first inferior transgenic heterozygotes have to be generated. The synthetic maternal toxin–zygotic antidote MEDEA system has, however, the advantage that the transgenes can be introduced *via* the males that show — due to the lack of maternal toxicity — normal Mendelian inheritance. Moreover, only males are necessary to actually inundate the wild-type population in order to spread the transgene combinations (Figure 2C). This is especially important for mosquito releases, because the release of female mosquitoes, which feed on humans, might actually increase temporarily the transmission of vector-borne diseases. This actually is also the drawback of the described two loci underdominance system in respect to transgene recall [8], as this requires the release of large quantities of females, which is probably not tolerable in a disease-threatened area.

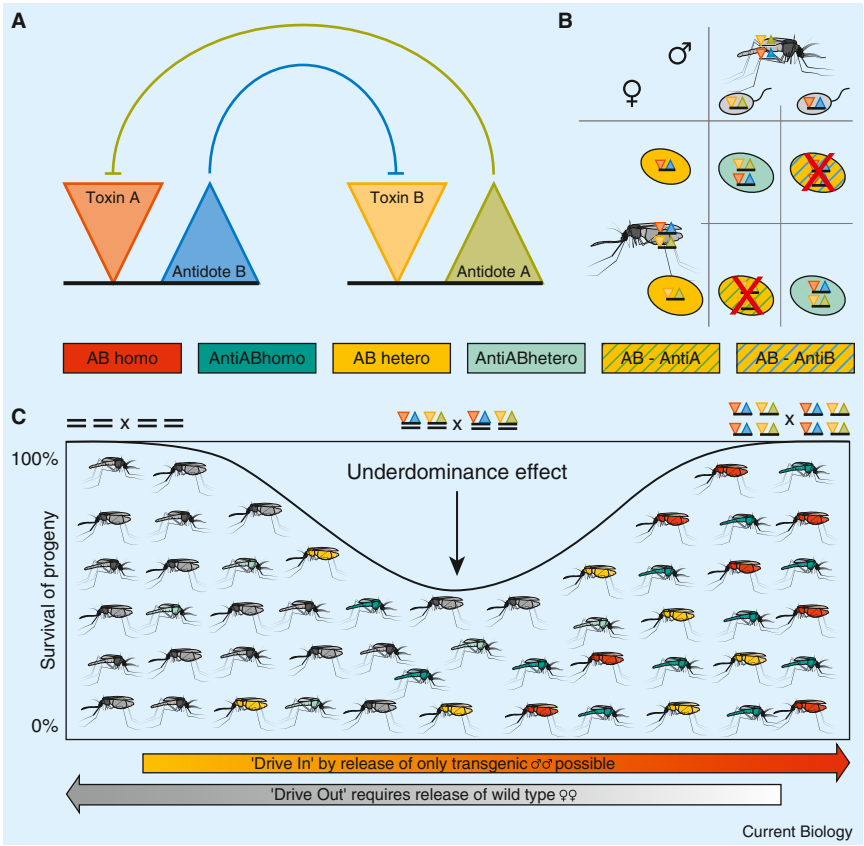

Figure 2. Gene drive by double-MEDEA systems.

(A) Double-MEDEA system [8], in which one transgene pair produces one maternal toxin (A) and the zygotic antidote (AntiB) for another maternal toxin (B) produced by a second transgene pair that also produces the zygotic antidote for the first toxin (AntiA). (B) In a single locus double-MEDEA system the two reciprocal maternal toxin–zygotic antidote transgene pairs are placed at the same position of homologous chromosomes. In this situation only the 50% trans-heterozygote progeny survives, while the homozygotes die (red X), as they miss one antidote [8]. In this scenario the homozygotes are at a disadvantage, which actually resembles a genetic overdominance system (heterozygote superiority). (C) In a two loci double-MEDEA system, the two reciprocal maternal toxin–zygotic antidote transgene pairs are placed at different positions in the genome (e.g. on different chromosomes). When the mother carries both transgene pairs and thus produces both toxins, both gene loci need to be inherited by the progeny for survival. This leads to an underdominance effect (heterozygote inferiority), since only 56% of progeny of a double heterozygote cross (middle) will survive, while 100% progeny of a wild type (left side) or a double homozygote cross (right side) will survive [8]. For the 'drive in' with the maternal toxin–zygotic antidote MEDEA system actually only males carrying the transgenes are necessary. However, to 'drive out' the MEDEA system again also females would have to be released in large numbers [8].

Hay and colleagues [8] also describe a single locus double-MEDEA system that acts as a genetic overdominance (heterozygote superiority) system [20], in which both homozygous genotypes have a lower fitness than the heterozygous genotype (Figure 2B). One disadvantage of the single locus system is that only half of the progeny survive, which might hamper mass rearing. Also the single locus system can be introduced into a population via male-only releases, which requires, however, a very high release ratio or releases over two consecutive generations [8]. This would serve as a local confinement factor for populations with moderate migration rates [7]. Moreover, the single locus system has two other advantages: first, the release of wild-type males into an established transgenic (heterozygous) population causes a dramatic population decline, which could be used as a mosquito control measure similar to SIT in addition to the refractoriness to disease transmission. Second, after such an induced

population decline only few males and females would have to be released to actually recall the transgenes and revert the mosquito population to wild type (drive out, reversion).

So far these systems have only been described in the non-pest insect model organism *Drosophila* [8], and it will take its time until similar strategies will have been successfully developed for human disease vectors. In addition, it will take enormous efforts by international regulators in collaboration with molecular entomologists, ecologists, and operational pest managers to develop clear regulatory frameworks for the safe release of such beneficial transgenic insects. Nevertheless, the principal concepts to establish transgenic refractoriness to malaria or dengue transmission in mosquitoes as well as to control a locally refined spread and to recall the transgenes are established, which nurtures the long-standing hope that insect transgenesis can indeed be employed for novel strategies to fight human vector-borne diseases.

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Chromatin: A Tail of Repression

Genetic evidence on the role of specific histone amino acids or their posttranslational modifications in metazoan development has been lacking. A recent study reports that fruit flies carrying histone H3 lysine 27 (H3K27) mutations have the same homeotic gene expression and developmental defects as mutations in the enzyme that trimethylates H3K27.

Danesh Moazed

The four canonical histones, H2A, H2B, H3, and H4, are among the most highly conserved eukaryotic proteins. Between cow and pea, all but 2 of the 103 amino acids in histone H4 are the same, and there are only 8 amino acid differences between the yeast and human H4 proteins. Histones package DNA into chromatin and in keeping with their extraordinary conservation play important roles in nearly all DNA transactions. That the post-translational modifications of histones play a central role in the regulation of chromatin structure and transcription is a basic tenant of current models of gene regulation. It may come as a surprise to many that in multicellular eukaryotes a direct demonstration of a role for a specific histone amino acid, or its modification, in gene activation or silencing was lacking until recently. In a paper published in *Science* last month [1], Muller, Herzig and colleagues now remedy this situation by demonstrating that a point mutation in lysine 27 of histone H3 (H3K27) fails to silence genes that are targeted by the Polycomb Repressive Complex 2 (PRC2), the methyltransferase that modifies H3K27.

The basic unit of chromatin is the nucleosome, which contains 147 base pairs of DNA wrapped twice around an octamer composed of four histones [2]. Histones contain a variety of posttranslational modifications, which are mostly but not exclusively concentrated on their amino termini. These modifications provide binding sites for proteins that mediate downstream functions, ranging from activation and repression of transcription to coordination of DNA damage repair. In addition, they affect the interaction of the positively charged histone tails with DNA, thereby regulating nucleosome stability [3]. In *Drosophila*, mammals, and many other multicellular organisms, the stable silencing of developmental regulators, such as the homeotic master regulators, outside of their proper